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Running Title: NA cessation for HBV reactivation

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Conflict of interest: All authors declare that we do not have anything to disclose regarding funding or conflict of interest with respect to this study.

Author contributions: Study desingn and discussion: A.T., K.K., K.K., H.E., K.N., and M.M. Clinical data collection: A.T., K.K., K.K., H.E., N.O., R.K., S.U., and M.E. Statistical analysis: A.T. and M.E. Manuscript writing: A.T. All authors read and approved the final manuscript.

Disclaimers: This paper has not been published or presented elsewhere in part or in entirety, and is not under consideration by another journal.

Funding: This study was supported by a Research Grant from the Japan Agency for Medical Research and Development. (e-rad ID number: 18950494).

Mandates Data Sharing: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abbreviations: HBsAg, hepatitis B surface antigen; HBcrAg, HB core related antigen; Anti-HBs, antibodies to hepatitis B surface antigen; CH, chronic hepatitis; EOT, end of treatment; NA, nucleos(t)ide analogue; ML, malignant lymphoma; HSCT, hematopoietic stem cell transplantation; RA, rheumatoid arthritis.

Abstract

Background and aim: Preventive or on-demand nucleos(t)ide analog (NA) therapy can prevent severe hepatitis related to hepatitis B virus reactivation (HBV-R). However, it is unclear if NA can be safely stopped in such patients after cytotoxic therapies or during immunosuppressive therapies.

Patients and methods: We retrospectively evaluated 133 patients who initiated NA therapy between 2007 and 2018. A total of 103 patients were positive for HBV surface antigen (HBsAg) at baseline, and NA therapy was started before cytotoxic or immunosuppressive therapy (preventive group). Thirty patients with resolved HBV infection were treated with NA therapy after HBV reactivation (on-demand group). Virological relapse was defined as a serum HBV DNA level >20 IU/mL.

Results: NA therapy was stopped in 12 (12%) patients (preventive group), and in 16 (53%) patients (on-demand group). After the cessation of NA therapy, the cumulative rates of relapse were 36% and 39% at 12 and 24 months, respectively. High levels of HBsAg both at baseline and at the cessation of NA therapy were related to the occurrence of relapse. Relapse did not occur in patients with HBsAg levels <20 IU/mL (preventive group). HBV relapse occurred in five (33%) patients in the on-demand group. Relapse occurred only in anti-HBs-negative patients at the cessation of NA therapy. There were no cases of hepatitis flare after the cessation of NA therapy.

Conclusion: HBsAg predicted HBV relapse after the cessation of NA therapy in HBsAg-positive patients. Anti-HBs could be a predictive marker for NA therapy cessation in patients with resolved HBV.

Electronic word count: 249

Key Words: Anti-HBs; HBsAg; nucleos(t)ide analog; relapse

1 | INTRODUCTION

Hepatitis B virus reactivation (HBV-R), which is frequently induced by cytotoxic or immunosuppressive therapy, can potentially lead to serious outcomes.[1,2] HBV surface antigen (HBsAg)-positive patients are at high risk of HBV-R, and should receive nucleos(t)ide analog (NA) therapy before the initiation of immunosuppressive or cytotoxic therapy.[3,4] HBsAg-negative and anti-HBc-positive patients with a resolved HBV infection are at a lower risk of HBV-R than HBsAg-positive patients, and depending on their clinical situation and feasibility of close monitoring, they can begin anti-HBV prophylaxis or monitoring with the intent of initiation of on-demand anti-HBV therapy at the first sign of HBV-R.[3,4] These strategies work well to prevent fatal hepatitis induced by HBV-R. The patients who were HBsAg-positive at baseline included healthy carriers with lower levels of HBV DNA for whom anti-HBV therapy could be stopped after immunosuppressive or cytotoxic therapy. Regardless of HBV-R, some patients with resolved HBV infection received NA preventive therapy before receiving B cell-depleting agents, including rituximab, ofatumumab, natalizumab, alemtuzumab, and ibritumomab.[3,4] The WHO estimated that 257 million people were living with a chronic hepatitis B infection worldwide in 2015.[5] It was speculated that the same or a greater number of people had a past HBV infection. To care for HBV-related patients according to current guidelines, the total cost for HBV DNA testing and NA treatment will be high. Recently, the high-sensitivity HBsAg assay may be replaced by an HBV DNA test for the

diagnosis of HBV-R.[6] Cessation of preventive therapy is one strategy used in the care of these patients. However, it is recommended that NA administration should be continued for 1 year after the cessation of cytotoxic therapy.[7] There have been no comprehensive studies of patients after the end of antiviral prophylaxis. In the present study, we evaluated the clinical features of patients following the initiation of NA preventive therapy for HBV-R, in particular patients in whom NA treatment was stopped.

2 | PATIENTS AND METHODS

2.1 | Study Design

This was a multi-center retrospective observational study.

2.2 | Patients

A total of 133 patients who began NA treatment for HBV-R between 2005 and 2018 were enrolled (Table 1). The observation periods ranged from 1 to 148 months (median 47 months). Of these patients, 103 were HBsAg-positive at baseline on the commencement of cytotoxic or immunosuppressive therapy, including 44 females and 59 males, ranging in age from 17 to 86 years old. Preventive NA administration was started before or concomitantly with cytotoxic or immunosuppressive therapy. The HBV viral load ranged from 1.9 to 8.2 log IU/mL; HBV DNA was below the limit of detection in 17 patients. The original diseases were malignant lymphoma (*n* = 13), hematopoietic stem cell transplantation (HSCT) for hematological malignancy (*n* = 1), kidney

transplantation (*n* = 3), rheumatoid arthritis (*n* = 24), chemotherapy for solid malignant diseases (*n* = 49), and immunosuppressive therapy for benign diseases (*n* = 13). At baseline, 2 (2%) patients were immune-tolerant chronic hepatitis B (CHB), and 86 (83%) patients were inactive chronic hepatitis B (CHB), while 15 (15%) patients were diagnosed with immune-active CHB and were recommended for anti-HBV therapy regardless of HBV reactivation.[4] The remaining 30 patients were HBsAg-negative and anti-HBc-positive at baseline. On-demand anti-HBV therapy was started on the detection of HBV-R. The original diseases were malignant lymphoma (*n* = 9), HSCT for hematological malignancy (*n* = 9), kidney transplantation (*n* = 3), rheumatoid arthritis (*n* = 7), and chemotherapy for solid malignant diseases (*n* = 2). No patient received an HB vaccine.

2.3 | **Assay**

HBsAg was quantified by chemiluminescent enzyme immunoassay (Lumipulse HBsAg HQ; Fujirebio Inc., Tokyo, Japan). HBsAg positivity was defined as ≥ 0.005 IU/mL. Serum samples were tested for anti-HBc antibody and antibodies to HBsAg (anti-HBs) by chemiluminescent enzyme immunoassays (CLEIA; Fujirebio Inc., Tokyo, Japan). Anti-HBs positivity was defined as an anti-HBs titer >10 mIU/mL. Serum HB core-related antigen (HBcrAg) levels were measured using a CLEIA HBcrAg assay kit with a fully automated Lumipulse System analyzer (Fujirebio) as described previously.[8] The HBcrAg

concentration was calculated based on a standard curve generated using recombinant pro-HBeAg. The immunoreactivity of pro-HBeAg at 10 fg/mL was defined as 1 U/mL. We expressed HBcrAg in terms of log U/mL, with the quantitative range set at 3.0 – 6.8 log U/mL. HBV DNA was examined once every 3 months using a real-time polymerase chain reaction (PCR)-based method (COBAS TaqMan PCR; Roche Diagnostics, Tokyo, Japan).[9] The quantitative range of the real-time PCR assay was 20 – 200000000 IU/mL (1.3 – 8.2 log IU/mL). The relapse risk score for chronic hepatitis B was calculated as described previously.[10] In details, the HBV relapse risk score was given by the total of the HBsAg and HBcrAg scores. The HBsAg score was determined as follows: HBsAg load (IU/mL) < 80, 0; ≥ 80 and < 800, 1; and ≥ 800, 2. The HBcrAg score was determined as follows: HBcrAg load (U/mL) < 3.0 log, 0; ≥ 3.0 log and < 4.0 log, 1; and ≥ 4.0 log, 2.

2.4 | Definition of HBV relapse and hepatitis flare after NA cessation

Virological relapse was defined as a serum HBV DNA level >20 IU/mL[7] and hepatitis flare was defined as fivefold of the upper normal limit of ALT.

2.5 | Statistical analysis

Data analyses were conducted using JMP software (ver. 9.0; SAS Institute, Cary, NC). Differences between groups were evaluated by Wilcoxon's two-sample test for numerical variables or Fisher's exact test for categorical variables. In the two-tailed tests, p <0.05 was taken to indicate statistical

significance. Kaplan-Meier curves were used to analyze the cumulative HBV relapse rate after the cessation of NA therapy.

2.6 | Ethical considerations

This study protocol complied with the ethical guidelines of the Declaration of Helsinki 1975 (2005 revision) and was approved by the Ethics Committee of Osaka City University Graduate School of Medicine.

3 | RESULTS

3.1 | Long-term outcomes of patients with NA therapy

For all patients, entecavir administration was started as the first preventive therapy for HBV-R. In two patients with HBeAg at baseline, entecavir was replaced with tenofovir alafenamide. During preventive antiviral therapy with NAs, the HBV DNA levels were <20 IU/mL in all patients, and there were no incidences of hepatitis flare. Twenty-seven patients died due to progression of the original disease, and not liver diseases associated with HBV-R.

3.2 | Comparison of the clinical characteristics of the NA continuation and NA cessation groups

NA therapy was stopped at the discretion of the attending physician in 12 (12%) of 103 patients with HBsAg at baseline and 16 (53%) of 30 patients with on-demand therapy (Table 2). The former 12 patients consisted of 6 undergoing chemotherapy for solid cancer, 3 receiving steroid therapy for benign disease, 2

with rituximab combined chemotherapy for malignant lymphoma, and 1 with kidney transplantation (Table 3). After the completion of cytotoxic or immunosuppressive therapy, NA administration was stopped in 12 patients with inactive CHB. The median duration of NA therapy was 24 months. The HBsAg level and HBV DNA viral load at baseline were significantly lower in patients who stopped NA therapy compared to patients who continued NA therapy. Among them, HBsAg to anti-HBs seroconversion occurred during NA therapy in two patients; one with malignant lymphoma and the other with kidney transplantation.

Among the 30 patients with on-demand therapy, NA was stopped in 16 patients consisting of 7 with HSCT, 4 with rituximab combined chemotherapy for malignant lymphoma, 4 with rheumatoid arthritis, and 1 with kidney transplantation (Table 3). Immunosuppressive therapy without biologics was continued for four HBsAg-negative patients with rheumatoid arthritis after the cessation of NA therapy. There was a significant difference in the anti-HBs titer at baseline between the NA continuation and NA cessation groups.

3.3 | Risk factors for HBV relapse after NA cessation

The cumulative rates of virological relapse were 36% and 39% at 12 and 24 months, respectively (Figure 1). There were no instances of later HBV relapse. In details, HBV relapse occurred in 5 of 12 (41%) patients with preventive therapy during 12 - 43 months observation following the cessation of NA therapy (Table 4). Compared to the non-relapse group, the HBsAg levels at

baseline and at the end of treatment (EOT) were significantly higher in the HBV relapse group (p <0.01); relapse did not occur in patients with an HBsAg level <20 IU/mL at the EOT. In addition, the relapse risk score was significantly different between the two groups (p <0.01).

HBV relapse occurred in 6 (38%) of 16 patients with on-demand therapy during 10 – 119 months of observation following the cessation of NA therapy (Table 4). The anti-HBs titers at baseline were significantly higher in the HBV non-relapse group than in the relapse group (p = 0.01). In addition, no relapse occurred in patients positive for anti-HBs at the cessation of NA therapy (p <0.01). No difference in the duration of NA therapy was observed after stopping the cytotoxic or immunosuppressive treatment between patients with and without relapsed HBV. However, HBV relapse was seen in the on-demand group patients treated with NA for less than 20 months after stopping cytotoxic or immunosuppressive therapy.

3.4 | Outcome in patients with HBV relapse

NA retreatment was started in 5 of 11 patients with HBV relapse. These five patients had received on-demand therapy; after the cessation of NA therapy, immunosuppressive therapy was continued in two patients with rheumatoid arthritis and in one with kidney transplantation. Entecavir was restarted at the detection of an HBV DNA level ≥20 IU/mL. Another two cases showed interesting clinical courses (Figure 2). Case 1 was a 64-year-old male patient treated with rituximab combined chemotherapy for malignant lymphoma. On-

demand NA therapy was started during chemotherapy. NA was stopped 1 year after the end of chemotherapy. At the time, the HBV DNA, HBsAg, HBcrAg, and anti-HBs levels were below the limit of detection. Five months after the cessation of NA therapy, the patient's HBV DNA level increased to ≥2000 IU/mL. NA treatment was started again and the HBV DNA level decreased rapidly to below the limit of detection with an increasing anti-HBs titer. After the second cessation of NA therapy, HBV relapse did not occur during 5 years of follow-up. Case 2 was a 51-year-old male patient who underwent HSCT for acute myeloid leukemia. Entecavir was stopped 2 years after the end of on-demand therapy. At the time, the HBV DNA, HBsAg, HBcrAg, and anti-HBs levels were below the limit of detection. The patient's HBV DNA levels increased to 3.6 log IU/mL 7 months after NA cessation. NA therapy was restarted and the HBV DNA level decreased to below the limit of detection with an increasing anti-HBs titer. After the second cessation of NA therapy, HBV did not relapse during 1 year of follow-up.

In six patients without NA retreatment, cytotoxic or immunosuppressive therapy had been completed before NA cessation and the HBV viral levels were continuously <2000 IU/mL after relapse. Hepatitis flare did not occur in all 11 patients with HBV relapse.

4 | DISCUSSION

To our knowledge, this is the first study showing the outcome of patients with preventive NA therapy with long-term observation. No fatal events associated with HBV-R occurred in either the 103 HBsAg-positive patients or 30 patients with a resolved HBV infection. NA therapy was stopped in 12 patients with HBsAg at baseline and 16 patients with on-demand therapy without hepatitis flare. However, the HBV DNA levels increased to ≥20 IU/mL in 11 (39%) of 28 patients after the cessation of NA therapy; NA retreatment was necessary for 5 patients with HBV relapse, and the other 6 patients finally became healthy HBV carriers with only monitoring. In addition, two patients with retreatment achieved high anti-HBs titers, and HBV relapse did not occur after the second NA cessation. Finally, NA therapy was safely stopped in 25 (19%) of 133 patients in the present study.

NA cessation was performed after the end of cytotoxic or immunosuppressive therapy, except in four patients with rheumatoid arthritis. In the preventive group, NA was stopped only in patients with a low viral load at baseline. NA therapy was continued in healthy carriers with a high viral load at baseline after the completion of short-term steroid therapy for benign disease (sudden deafness and retrobulbar optic neuritis; Table 3). There was no evidence to support a need for continuous NA therapy in such cases.

With regard to safety after the cessation of NA therapy, a systematic review suggested that an HBsAg level <100 IU/mL at the EOT may be a useful marker

for deciding when to stop NA therapy in patients with chronic hepatitis B.[11] The Japan Society of Hepatology guidelines proposed that a relapse risk score by combined HBsAg and HBcrAg could be a predictive marker for safe discontinuation of NA therapy.[7] A recent study showed an EOT HBsAg level <40 IU/mL was optimal for the cessation of NA therapy.[12] However, there have been no reports regarding clinical markers for the cessation of NA therapy in patients under HBV-R management. In the present study, seven patients with an EOT HBsAg level <20 IU/mL continued to have an HBV DNA level <20 IU/mL for more than 19 months. Thus, it will be necessary to evaluate greater numbers of patients who have stopped NA therapy.

Several reports have suggested that anti-HBs could be a potential marker for predicting HBV-R in patients with a resolved HBV infection.[13–15] In addition, clinical trials for HSCT suggested that amplification of anti-HBs by HB vaccination had the potential to prevent HBV-R.[16,17] In the present study, viral relapse did not occur in patients whose anti-HBs became positive on the cessation of NA therapy. In particular, in two patients with relapse after the first NA cessation, HBV did not relapse after achieving anti-HBs seroconversion. Amplifying anti-HBs by vaccination could be a promising approach to stop NA. The results of the present study suggest the additional usage of anti-HBs in HBV-R management. However, HBV with a surface antigen escape mutation may emerge or reactivate in patients with high titers of anti-HBs.[18,19] Therefore,

further study is necessary to evaluate the role of anti-HBs in the management of HBV-R.

This study has some limitations. First, the number of patients who stopped NA therapy was small. Non-hepatologists cared for one-third of the patients to treat comorbidities other than HBV infection. It was difficult to determine NA cessation in these cases. Second, the enrolled patients had varied clinical backgrounds, including different target diseases for cytotoxic or immunosuppressive agents. The outcome after NA cessation was dependent on many factors, not only HBV infection. Finally, HBV-R management in some enrolled patients was not conformed the guideline.[3,4,7] The interval between HBV DNA monitoring in the on-demand group and the duration of NA therapy after stopping cytotoxic or immunosuppressive treatment depended on the decision of the doctor in charge. A prospective study will be necessary to validate the present results.

In conclusion, NA preventive therapy can be ceased safely in patients after cytotoxic or immunosuppressive therapies. HBsAg and relapse risk score at the EOT might predict HBV relapse in HBsAg-positive patients at baseline. In patients with resolved HBV at baseline, anti-HBs could be a predictive marker for NA cessation.

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Figure Legends

FIGURE 1 Cumulative rate of HBV relapse after the cessation of nucleoside analog treatment. HBV relapse did not occur after more than 2 years of observation. The median observation period after NA cessation was 35 months (10 – 119 months).

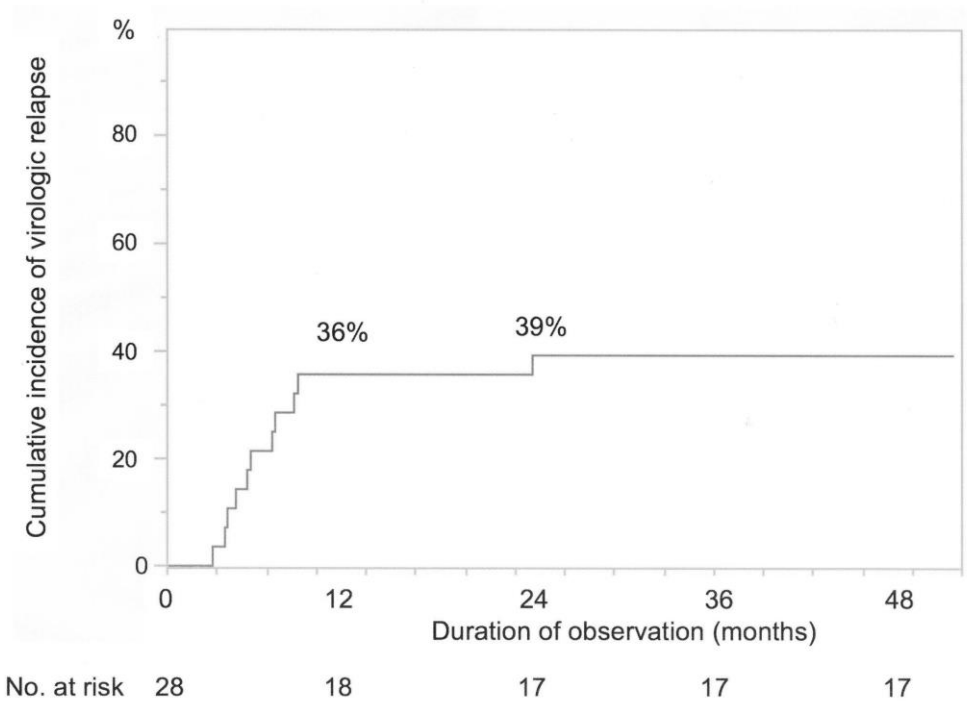


FIGURE 2 Clinical courses of two patients with anti-HBs seroconversion after HBV relapse.

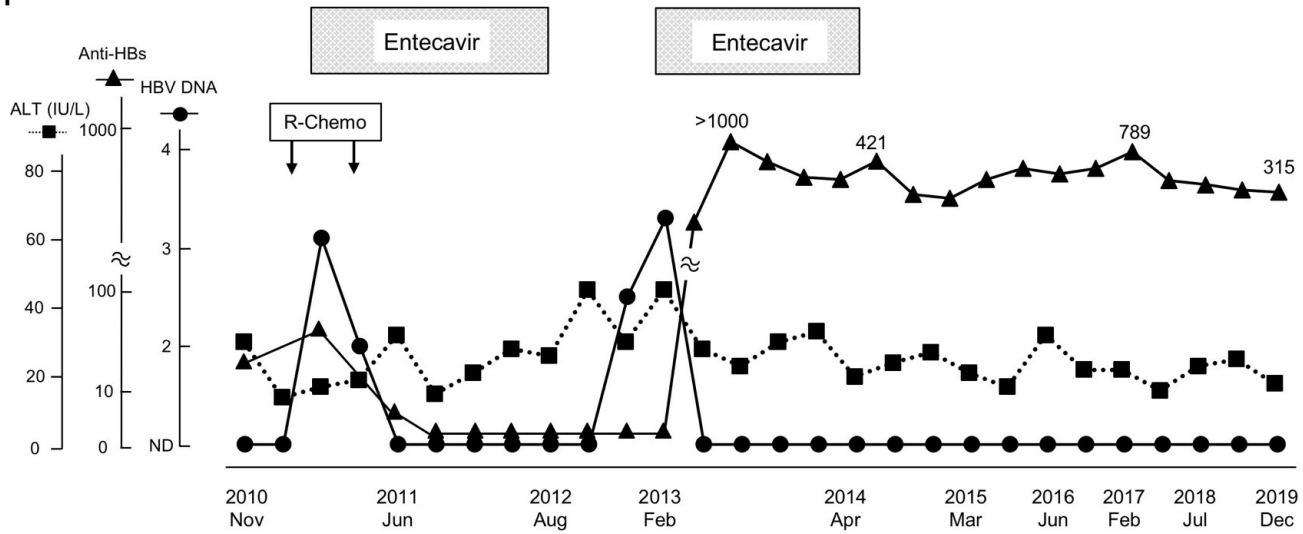
Case 1: A 64-year-old male patient treated with rituximab combined chemotherapy for mantle cell lymphoma.

Case 2: A 51-year-old male patient treated with cord blood transplantation (CBT) for acute myeloid leukemia.

In both patients, HBV DNA increased after the first cessation of entecavir. However, after achieving anti-HBs seroconversion, HBV did not relapse during observation from the second NA cessation.

Figure 2

Case 1



Case 2

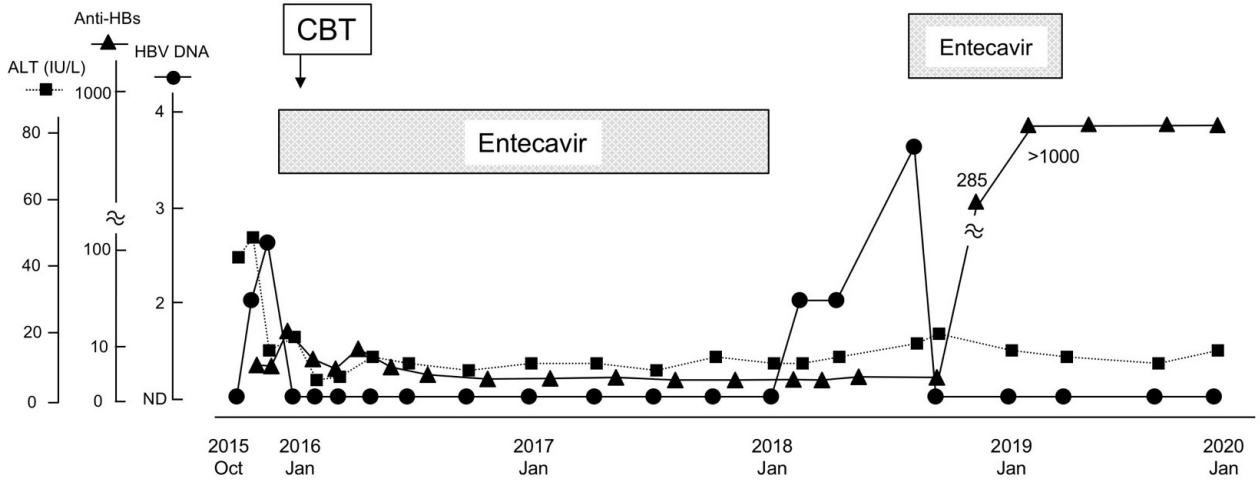


TABLE 1 Clinical characteristics of patients with NA preventive therapy

	HBsAg-positive at baseline Preventive therapy	HBsAg-negative at baseline On-demand therapy
Number of patients	103	30
Age* (years)	63 (17 – 86)	61 (43 – 85)
Sex: M/F	59/44	15/15
ML/HSCT/Kidney transplantation/RA/Chemotherapy/Other**	13/1/3/24/49/13	9/9/3/7/2/0
HBsAg* (IU/mL) at baseline	1131 (0.011 – 3030)	-
HBV DNA* (log IU/mL) at baseline	3.1 (<1.3 – 8.2)	-
Anti-HBs* at baseline (mIU/mL)	-	12.95 (0 – 799)
HBV status	Immune-active CHB: 15 (15%) Immune-tolerant CHB: 2 (2%) Inactive CHB: 86 (83%)	Resolved HBV: 30 (100%)
Observation period* (months)	49 (1 – 148)	81 (6 – 145)
Death: n (%)	25 (24%)	2 (7%)

*Continuous variables were shown median (range). **Other, benign diseases treated with steroids.

Anti-HBs, anti-hepatitis B surface antigen antibody; HBsAg, hepatitis B surface antigen; HSCT, hematopoietic stem cell transplantation; CHB, chronic hepatitis B; ML, malignant lymphoma; NA, nucleos; RA, rheumatoid arthritis(t)ide analog.

TABLE 2 Comparison of clinical characteristics between patients with NA continuation and NA cessation

	NA continuation	NA cessation	*p*-value
Preventive therapy			
Number of patients	91	12	
Age* (years)	63 (17 – 86)	61 (44 – 81)	0.72
Sex: M/F	52/39	7/5	0.94
ML/HSCT/Kidney transplantation/RA/Chemotherapy/ Other**	11/1/2/24/43/10	2/0/1/0/6/3	0.26
HBsAg* (IU/mL) at baseline	925 (0.01 – 4350)	17.51 (0.015 – 3030)	0.01
HBV DNA* (log IU/mL) at baseline	3.2 (<1.3 – 8.0)	1.0 (<1.3 – 3.0)	<0.01
HBV status at baseline: Immune-active CH	15 (16%)	0 (0%)	0.13
NA treatment period* (months)	41 (1 – 144)	24 (10 – 34)	0.045
Duration of the NA therapy after the completion of cytotoxic or immunosuppressive therapy (months)	0*** (0-73)	19 (7-29)	<0.01
Death: n (%)	25 (27%)	0 (0%)	
On-demand therapy			
Number of patients	14	16	
Age* (years)	67 (43 – 85)	60.5 (44 – 81)	0.43
Sex: M/F	43990	44081	0.46
ML/HSCT/Kidney transplantation/RA/Chemotherapy	5/2/2/3/2	4/7/1/4/0	0.59
Anti-HBs* (mIU/mL) at baseline	4.4 (0.0 – 30.2)	26.9 (0.0 – 799)	0.01
HBV DNA* (log IU/mL) at the peak of reactivation	3.1 (1.4 – 6.0)	3.0 (1.8 – 5.6)	0.88
NA treatment period* (months)	27 (1 – 100)	25 (5 – 65)	0.56
Duration of the NA therapy after the completion of cytotoxic or immunosuppressive therapy (months)	0*** (0-100)	16 (5-65)	<0.01
Death: n (%)	2 (14%)	0 (0%)	

*Continuous variables were shown median (range). **Others, benign diseases treated with steroids. ***0; The duration ‘0’ indicated that cytotoxic or immunosuppressive therapy was continued.

Anti-HBs, anti-hepatitis B surface antigen antibody; HBsAg, hepatitis B surface antigen; HSCT, hematopoietic stem cell transplantation; Immune-active CH, immune-active chronic hepatitis; ML, malignant lymphoma; NA, nucleos(t)ide analog; RA, rheumatoid arthritis.

TABLE 3 Clinical backgrounds of 28 patients in whom NA treatment was stopped

Patient Number	Age (years)	Gender	Original Diseases	Treatment	HBV status at baseline
1	57	F	Breast Cancer	Docetaxel + Cyclophosphamide	Inactive CHB
2	66	F	Colon Cancer	Folinate + Tegafur + Uracil	Inactive CHB
3	69	M	Esophageal Cancer	FP + Radiation	Inactive CHB
4	59	M	Lung Cancer	CDDP + VNR + Radiation	Inactive CHB
5	81	F	ML	R-CHOP	Inactive CHB
6	57	M	Retrobulbar optic neuritis	Prednisolone	Inactive CHB
7	57	M	Sudden deafness	Betamethasone	Inactive CHB
8	44	M	Renal failure	Kidney transplantation	Inactive CHB
9	78	F	Colon Cancer	Oxaliplatin + Capecitabine	Inactive CHB
10	63	F	Esophageal Cancer	FP	Inactive CHB
11	51	M	ML	R-CHOP	Inactive CHB
12	67	M	Sudden deafness	Prednisolone	Inactive CHB
13	53	M	ML	R-CHOP	Resolved HBV infection
14	60	M	ML	R-CHOP	Resolved HBV infection
15	46	F	Myelodysplastic syndrome	HSCT	Resolved HBV infection
16	55	M	AML	HSCT	Resolved HBV infection
*17	64	M	ML	R-HyperCVAD+MA	Resolved HBV infection
18	44	M	HSCT	HSCT	Resolved HBV infection
19	73	F	RA	Adalimumab	Resolved HBV infection
20	81	M	RA	Tocilizumab + methotrexate	Resolved HBV infection
21	61	M	Renal failure	Kidney transplantation	Resolved HBV infection
22	78	F	RA	Tocilizumab	Resolved HBV infection
23	58	F	RA	Prednisolone + methotrexate	Resolved HBV infection
**24	51	M	AML	HSCT	Resolved HBV infection
25	62	F	ML	HSCT	Resolved HBV infection
26	64	F	ML	R-CHOP	Resolved HBV infection
27	47	F	ALL	HSCT	Resolved HBV infection
28	71	M	AML	HSCT	Resolved HBV infection

*17, Case 1 in Figure 2; **24, Case 2 in Figure 2.

ML, Malignant lymphoma; RA, Rheumatoid arthritis; AML, Acute myeloid leukemia; ALL, Acute lymphocytic leukemia; R-CHOP, Rituximab + Cyclophosphamide + Doxorubicin + Vincristine + Prednisolone; CDDP, Cisplatin; VNR, Vinorelbine; FP, Fluorouracil + Cisplatin; HSCT, hematopoietic stem cell transplantation; Inactive CHB, inactive chronic hepatitis B.

TABLE 4 Factors associated with HBV relapse after NA therapy cessation

	With HBV relapse	Without HBV relapse	*p*-value
Preventive therapy			
Number of patients	5	7	
Age* (years)	57 (51 – 78)	66 (44 – 81)	0.46
Sex: M/F	3/2	5/2	0.28
ML/HSCT/Kidney transplantation/RA/Chemotherapy/ Other**	1/0/0/0/3/1	1/0/1/0/3/2	0.79
HBsAg* (IU/mL) at baseline	1271 (1010 – 3030)	2.29 (0.02 – 19.5)	< 0.01
HBV DNA* (log IU/mL) at baseline	2.5 (<1.3 – 3.0)	0 (<1.3 – 2.0)	0.04
HBsAg* (IU/mL) at NA cessation	545.3 (94.1 – 1507)	0.18 (0 – 11.1)	< 0.01
HBcrAg* at NA cessation < 3.0 log U/mL	4 (80%)	6 (86%)	0.79
Relapse Risk Score*: 0/1 – 2/3 – 4/NT	0/5/0/0	6/0/0/1	< 0.01
NA treatment period* (months)	24 (14 – 30)	24 (10 – 34)	0.94
Duration of the NA therapy after the completion of cytotoxic or immunosuppressive therapy (months)	18 (9-27)	19 (7-29)	0.57
Observation period* after NA cessation (months)	28 (12 – 37)	34 (19 – 43)	0.37
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On-demand therapy			
Number of patients	6	10	
Age* (years)	59.5 (51 – 73)	61 (44 – 81)	0.96
Sex: M/F	4/2	5/5	0.52
ML/HSCT/Kidney transplantation/RA/Chemotherapy	2/1/1/2/0	2/6/0/2/0	0.38
Anti-HBs* at baseline (mIU/mL)	18.6 (1.6 – 26.9)	51.8 (0.0 – 799)	0.01
Anti-HBc* at baseline (mIU/mL)	99.5 (6.9 – 146.4)	76.3 (3.9 – 97.9)	0.46
HBV DNA* (log IU/mL) at peak of reactivation	2.75 (2.2 – 4.7)	3.3 (1.8 – 5.6)	0.3
Anti-HBs* at NA cessation (mIU/mL)	0.45 (0.0 – 8.0)	36.75 (14 – 1000)	< 0.01
NA treatment period* (months)	25 (16 – 45)	19 (5 – 65)	0.75
Duration of the NA therapy after the completion of cytotoxic or immunosuppressive therapy (months)	15 (6-20)	26 (5-65)	0.44
Observation period* after NA cessation (months)	45 (18 – 119)	41 (10 – 92)	0.33

*Continuous variables were shown median (range).**Others, benign diseases treated with steroids.

Anti-HBs, anti-hepatitis B surface antigen antibody; HBcrAg, HB core-related antigen; HBsAg, hepatitis B surface antigen;

HSCT, hematopoietic stem cell transplantation; ML, malignant lymphoma; NA, nucleos(t)ide analog; RA, rheumatoid arthritis